

MINIREVIEW

The RACK1 Scaffold Protein: A Dynamic Cog in Cell Response Mechanisms

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Our understanding of the enigmatic receptor for activated C-kinase 1 (RACK1) protein has increased dramatically in recent years from its original identification as an anchoring protein for protein kinase C (PKC) (Ron et al., 1994a). By virtue of its ability to coordinate the interaction of key signaling molecules, RACK1 is becoming widely perceived as playing a central role in critical biological responses, such as cell growth. RACK1 is a 36-kDa protein (SwissProt accession no. P25388) containing seven internal Trp-Asp 40 (WD40) repeats (Fig 1A), with a consensus X^{6-94} -[GH-X²³⁻⁴¹-WD]^{N₄-8} (where N = number of WD repeats). It is homologous to the G protein β subunit, having 42% identity with many conserved amino acid substitutions. The WD repeats of RACK1 can be predicted to form a seven-bladed propeller structure (Sondek and Siderovski, 2001; Steele et al., 2001), with each blade made up of β -sheets as shown in crystallographic studies for G_{β} (Wall et al., 1995; Sondek et al., 1996). The WD repeat sequence of RACK1 is highly conserved in a diverse range of species, including plants (Kwak et al., 1997) and genetically malleable species such as *Drosophila melanogaster* and *Caenorhabditis elegans* (Bini et al., 1997). Positioning of RACK1 WD repeats is even maintained in the alga *Chlamydomonas reinhardtii* (Schloss, 1990), which diverged from the forerunners of the plant and animal kingdoms some 600 million to 1 billion years ago. This has prompted the suggestion that the biological function of RACK1 was established before this separation occurred (Neer et al., 1994). Indeed, RACK1 is ubiquitously expressed in the tissues of higher mammals and humans (Guillemot et al., 1989), including brain, liver, and spleen, suggesting that it has an important functional role in most, if not all, cells (Chou et al., 1999).

RACK1 was originally cloned from both a chicken liver cDNA library and a human B-lymphoblastoid cell line (Guillemot et al., 1989) and referred to as C12.3 or H12.3, respectively. The name RACK1 was adopted by the Mochly-Rosen group to describe its ability to bind activated PKC. This was because the rat gene product passed experimental criteria similar to those used to identify protein A-kinase anchoring proteins (Edwards and Scott, 2000). These criteria were originally established by Ron et al. (1994) and recently refined by Dorn and Mochly Rosen (2002): 1) injection of cells with purified RACK should block PKC-mediated cell processes. Similarly, 2) delivery of peptides into cells should block the interaction between a particular PKC isozyme and its RACK, and this should specifically impair a known cellular function of that isozyme. 3) Injection of peptides that induce an interaction between a particular PKC isozyme and its RACK should selectively activate that isozyme, and 4) RACK should bind PKC in the presence of PKC activators (Ron and Mochly-Rosen, 1994; Dorn and Mochly-Rosen, 2002).

The first report on the structure and genomic organization of a mammalian RACK was carried out on the porcine *RACK1* gene (Chou et al., 1999), which has almost 100% identity at the protein level with its vertebrate homologs. The *RACK1* gene promoter contains a number of transcription factor binding sites including serum response element, AP1, SP1, NF1, and YY1 (Chou et al., 1999). Binding of serum response factor to the serum response element is known to be essential for the transcription of certain genes in response to growth factors; accordingly, RACK1 expression was found to be up-regulated after serum stimulation (Chou et al., 1999). That the activity of the *RACK1* gene is controlled by growth-

ABBREVIATIONS: RACK1, receptor for activated C-kinase 1; PKC, protein kinase C; PH, pleckstrin homology; 3D, three-dimensional; PDE, phosphodiesterase; RAID, RACK1 interaction domain; GGL, G- γ -like; CHO, Chinese hamster ovary; DECA, 1,1'-decamethylenebis-4-amin-oquinolindinium diiodide (dequalinium); CFTR, cystic fibrosis transmembrane regulator; PKA, protein kinase A; NMDA, *N*-methyl-D-aspartate receptor; IGF, insulin-like growth factor; Cpc, cross-pathway-control; MAPK, mitogen-activated protein kinase; ECM, extracellular matrix; IL, interleukin; GM-CSF, granulocyte macrophage-colony stimulating factor; Stat, signal transducers and activators of transcription; IFN, interferon; AD, Alzheimer's disease; GST, glutathione S-transferase.

promoting extracellular stimuli suggests that RACK1 may have a generalized role in the cellular adaptation processes that occur during cell division.

Diversity of Protein Interactions with RACK1

RACK1 was originally found to interact with active “conventional” PKC isoforms, with PKCβII seemingly being the preferred binding partner (Ron et al., 1995; Csukai and Mochly-Rosen, 1999; Stebbins and Mochly-Rosen, 2001). Conventional PKCs (α, βI, βII, and γ) are calcium- and diacylglycerol-dependent protein kinases that are activated after the receptor-stimulated hydrolysis of plasma membrane phosphatidylinositol 4,5-bisphosphate, which yields both calcium and diacylglycerol elevation (Mellor and Parker, 1998). Conventional PKCs, such as PKCβII (Fig. 2A), have in common a regular organization of conserved protein domains (C1–4), interspaced with isoform-specific, variable regions (Banci et al., 2002). C2 regulatory regions are found in a diverse range of proteins in addition to PKC (Fig. 3B) and were the first protein domains identified capable of interacting with RACK1 in a calcium- and phosphatidyl serine-

dependent manner (Banci et al., 2002). The PKC family is also represented by calcium-independent, “novel” PKCs (δ, ε, η, θ, and μ) and the diacylglycerol- and calcium-independent (atypical) PKCs (ζ and λ). RACK1 has been reported to interact with novel PKCs, e.g., PKCε (Besson et al., 2002). These observations strongly support the notion that RACK1 may regulate cell processes other than those involving conventional PKCs.

Each PKC isoform displays distinct tissue and subcellular distributions (Mellor and Parker, 1998). However, the tissue distribution of RACK1 is not always the same as its favored PKC (Chou et al., 1999). This raises the possibility that RACK1 may be involved in cell processes that are independent of PKC signal transduction (Chou et al., 1999). Indeed, the accumulated data from a number of laboratories show that RACK1 interacts with a range of different cellular proteins and, as a result, may have diverse, even cell-type-specific, functions (Table 1).

The protein liaisons that involve RACK1 seem to fall into two broad categories: constitutive, as with the cyclic AMP-specific phosphodiesterase PDE4D5 (Yarwood et al., 1999), and stimulus-dependent, as with PKC. The full range of

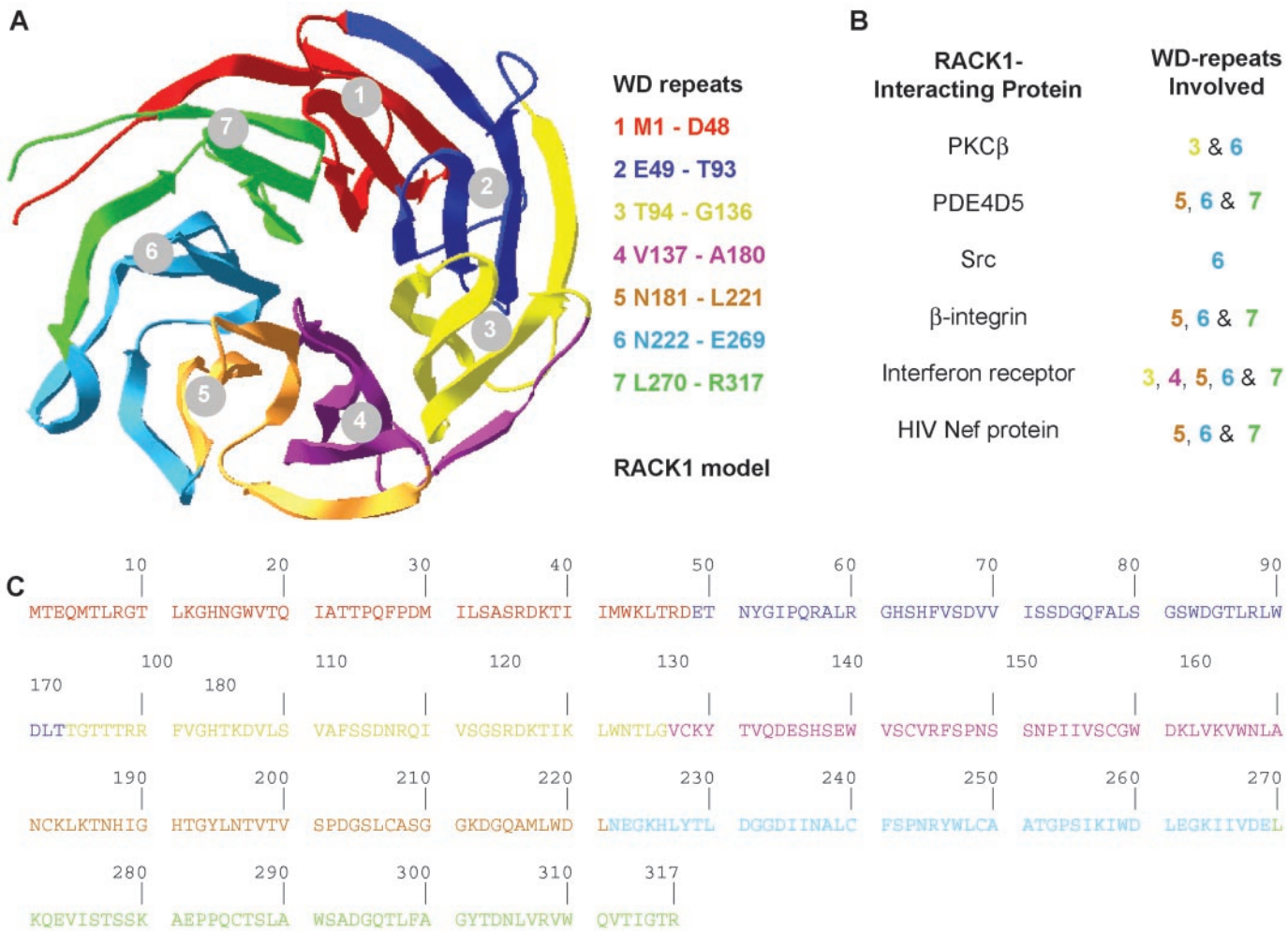


Fig. 1. Propeller blade and WD repeats in a RACK1 model. The 7-fold β-propeller structure of comparative modeled RACK1 is shown in A, with propeller blades numbered from the N terminus and color coding and residue numbering according to WD repeat sequences (Ron et al., 1994a). B, proteins whose interaction with RACK1 has been mapped to particular WD repeats within RACK1, indicated by numbering and color-coding. All protein graphics figures were prepared with Swiss-Pdb Viewer (Guex and Peitsch, 1997). C, linear amino acid sequence of human RACK1 (Swissprot GBLP_HUMAN; P25388) including residue numbers and WD-repeat color coding corresponding with those used in A.

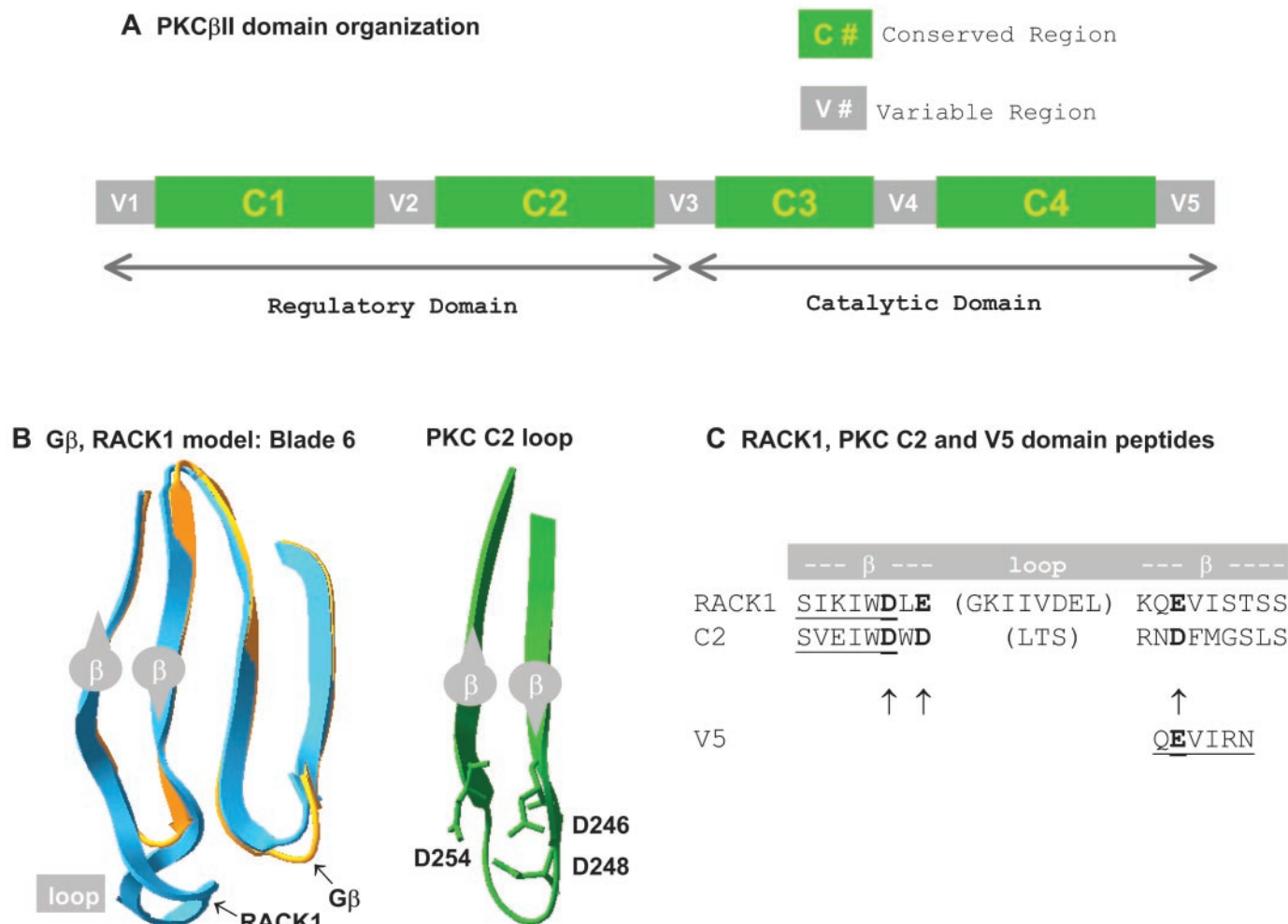


Fig. 2. Domain structure of PKC β II, RACK1, G β blade 6, and PKC C2 loop. A, the organization of regulatory, catalytic, conserved, and variable regions on a linear model of PKC β II is indicated by color shading and positional arrows. B, the four β -strands of RACK1 propeller blade 6 (blue) overlaid against the equivalent structure in G β (yellow) (Lambright et al., 1996). The major difference lies in the labeled loop, which connects the outer two strands of the blade with a substantial insert in RACK1 relative to G β . The equivalent two strands of PKC are shown in B, from protein databank coordinates 1a25 (Sutton and Sprang, 1998), and equivalence is defined through matching the SIKIWD RACK1 sequence with SVEIWD on the first of the two outer β -strands. Three residues involved in calcium ion binding in PKC are highlighted. The sequences for the displayed RACK1 and PKC strand-loop-strand structures are shown in C, with underlining at the SIKIWD and SVEIWD segments. The calcium ligands of PKC align with acidic residues in RACK1 (indicated by arrows). The QEVIRN sequence from the PKC V5 domain is also aligned to the second β -strand in RACK1, again with a common acidic residue.



Fig. 3. Similarities between GGL domains and RAID1. Shown are sequence similarities (top) between the GGL domains of G γ 1 and RGS11 and the N terminus (NT) of PDE4D5 as suggested by Sondek and Siderovski (2001). Conserved regions and semiconserved residues are highlighted with black and gray boxes, respectively. Residues within PDE4D5 NT that, when mutated to alanine, abrogate binding to RACK1 (Yarwood et al., 1999) are indicated with circles. Bottom, amino acid alignments of PDE4D5 NT with C2 domains of synaptotagmin, PI3K, PKC β II, and phospholipase A2 (cPLA2). Sequence similarities are indicated by black and gray boxes, and circles on the top line of the alignments denote amino acids in PDE4D5 NT critical for interaction with RACK1.

TABLE 1
RACK1 interacting proteins

RACK1 Interacting Proteins	References
PKCb: C2 and V5 domains	Ron et al., 1995; Stebbins and Mochly-Rosen, 2001
PDE4D5: RAID in unique N terminal region	Yarwood et al., 1999; Steele et al., 2001
Src family kinases: phosphotyrosine binding pocket of SH2 domain	Chang et al., 1998, 2001
PTPn catalytic region	Mourton et al., 2001
IL-3, IL-5, and GM-CSF receptors common β chain	Geijsen et al., 1999
PH domains of β -spectrin, β -dynamin, and p120GAP	Rodriguez et al., 1999; Koehler and Moran, 2001
GABA type A receptor: α 1 and β 1 subunits	Brandon et al., 1999
β -Integrin cytoplasmic domain	Liliental and Chang, 1998; Besson et al., 2002
Human type 1 interferon receptor: amino acids 300 to 346 of cytoplasmic domain	Croze et al., 2000
Pat1 Ran1 kinase with yeast RACK1 homologue Cpc2	McLeod et al., 2000
Pck2 with yeast RACK1 homologue Cpc2	Won et al., 2001
Epstein-Barr virus BZLF1 protein: transactivation domain	Baumann et al., 2000
Influenza virus M1 protein	Reinhardt and Wolff, 2000
P85 subunit of PI3 kinase and SHP-2	Kiely et al., 2002
HIV-1 Nef protein	Gallina et al., 2001
Adenovirus E1A protein	Sang et al., 2001
Type I interferon receptor and STAT1	Usacheva et al., 2001
<i>Helicobacter pylori</i> VacA cytotoxin	Hennig et al., 2001
NR2B subunit of the NMDA receptor	Yaka et al., 2002
NHERF1	Liedtke et al., 2002
IGF-1 receptor	Hermanto et al., 2002; Kiely et al., 2002

domains that allow protein partners to interact with RACK1 have yet to be determined; however, Src homology (SH2) domains (Chang et al., 1998) and pleckstrin homology (PH) domains (Rodriguez et al., 1999; Koehler and Moran, 2001) have been identified as possible candidates. PH domains are protein modules of 100 to 120 amino acids, best known for their ability to bind phosphoinositides (Lemmon et al., 2002). SH2 domains are also modular protein motifs of about 100 amino acids that interact with phosphotyrosine residues on target proteins (Pawson et al., 2001). The fact that different sorts of protein domain can interact with RACK1 suggests that RACK1 has multiple docking sites. In addition, the individual blades of the RACK1 β -propeller may be able to direct association with specific protein classes (Fig. 1B). The observation that PH domains and activated PKC can bind

concomitantly to RACK1 indicates that RACK1 indeed has multiple, independent protein binding sites (Rodriguez et al., 1999).

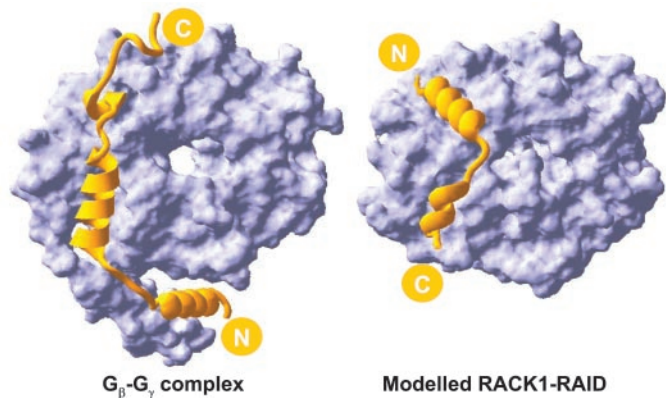


Fig. 4. Comparison of the $G\beta$ - $G\gamma$ structure and a RACK1-RACK1 interacting domain 1 (RAID) model. The crystallographic $G\beta$ - $G\gamma$ complex [protein databank file 1got (Lambright et al., 1996)] is shown (left) alongside a modeled RACK1-RAID complex (right). The model, based on mutagenesis studies, suggests that RAID may bind in a similar overall location to $G\gamma$ on the propeller framework and, in terms of overall placement, is similar to a previously reported model (Sondek and Siderovski, 2001). However, consideration of both mutagenesis data (Steele et al., 2001) and potential charge complementarity between RAID and RACK1 suggests that, in contrast to the earlier model, the RAID polypeptide direction on the RACK surface could be reversed relative to that of $G\gamma$ on $G\beta$.

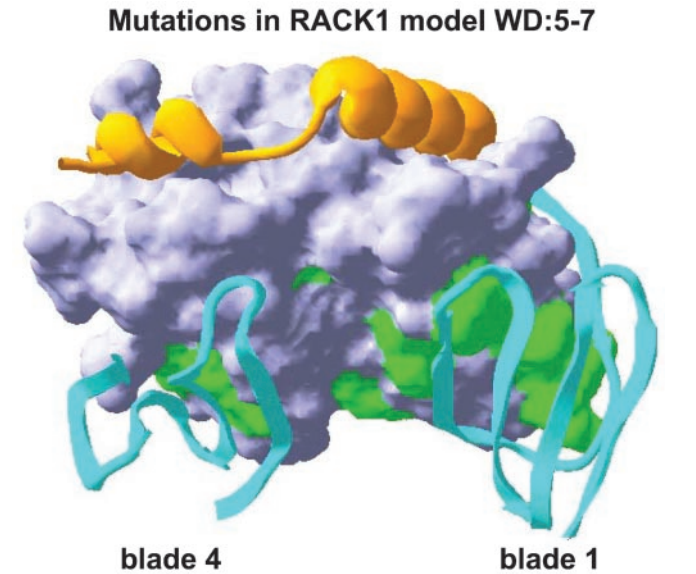


Fig. 5. Binding mutations in the RACK1 WD 5-7 model. A molecular surface is drawn for WD repeats 5 to 7 of the RACK1 model (excluding the N-terminal segment of WD repeat 5 that forms the outer β -strand of propeller blade 4). The molecule is turned to view partially into the interfaces with blades 1 and 4 of the 7-fold propeller (blue). RACK1 mutations that reduce RAID binding and have been isolated more than once (Steele et al., 2001) are highlighted in green on the molecular surface. Location of the displayed mutations at the blade interfaces for intact RACK1 indicates that their effect is not mediated directly through RACK1-RAID interactions. Because the mutations were isolated in the WD repeats 5 to 7 construct rather than the full propeller with its complement of blade interfaces, it is likely that stability of the individual blade structures is particularly sensitive to amino acid changes. Modeled RAID is shown as a yellow ribbon.

How Might Specific Proteins Interact with RACK1?

Before the full-spectrum of protein liaisons that involve RACK1 can be determined, it will necessary to examine how specific protein domains direct interaction with RACK1. To date, little has been done in this area; however, pioneering work on PKC-RACK1 interaction from the Mochly-Rosen laboratory and recent investigations into the binding of PDE4D5 to RACK1 have begun to give us a glimpse of how RACK1 may coordinate some of its interacting partners.

Interaction of PKC with RACK1 is thought not only to target PKC to appropriate intracellular locations but also to hold PKC in an active conformation (Dorn and Mochly-Rosen, 2002). This model is based on the premise that the PKC isoforms that are capable of interacting with RACK1 contain, within their primary amino acid sequence, a "pseudoRACK1" binding site (Ron et al., 1994b). It has been proposed that the pseudoRACK1 site directs auto-regulatory interactions allowing the pseudosubstrate site of PKC to interact with the substrate-binding site, thereby helping to maintain the enzyme in an inactive conformation (Ron et al., 1994b). Peptides have been discovered that can disrupt these interactions, thereby stabilizing the bound PKC in an open, active conformation (Ron et al., 1994b; Dorn and Mochly-Rosen, 2002). These peptides were derived from amino acid sequences within the C2 domain of PKC, which is thought to contain at least part of the RACK1 binding site, and from PKC-binding proteins such as annexin (Ron and Mochly-Rosen, 1995; Banci et al., 2002). One such example is the RACK1-derived peptide sequence DIINALCF, which is derived from amino acids 234 to 241, in WD 6, of RACK1. Not only can this peptide compete for the binding of PKC to RACK1 but also it can activate the enzyme *in vitro* and *in vivo* (Ron et al., 1994b; Ron and Mochly-Rosen, 1994). Peptides such as SIKIWD, which is derived from amino acids 255 to 260, WD 6, of RACK1, only represent a fraction of the PKC-binding site on RACK1 and are unable to stabilize PKC in an active conformation (Ron et al., 1994b; Dorn and Mochly-Rosen, 2002). The peptide SVEIWD, derived from amino acids 241 to 246 of the regulatory C2 region of PKC β , is a selective agonist of PKC β function and the corresponding structural region within the C2-domain of PKC β is thought to contribute significantly to the formation of the auto-regulatory region of that enzyme (Ron and Mochly-Rosen, 1995; Banci et al., 2002). Recent findings suggest that the binding sites for RACK1 on PKC β II are found not only in the C2 domain of the protein but also in the V5 region of the enzyme (Fig. 2A) (Stebbins and Mochly-Rosen, 2001). Accordingly, C2- and V5-containing peptide fragments also seem to inhibit the binding of RACK1 and PKC β (Ron et al., 1995; Stebbins and Mochly-Rosen, 2001). This suggests that the molecular interactions that occur between PKC and RACK1 are complex and may involve many points of contact between the two protein surfaces.

To try and put this peptide data in a structural context, we have constructed a comparative model of RACK1 using bovine transducin G β as the structural template (Fig. 1A). From this, we have identified an internal region that deviates markedly from the G β structure (Lambright et al., 1996). This region occurs in blade 6 of the RACK1 propeller and has a significantly longer inter- β -strand loop than the compara-

ble region in G β and may therefore have a role in determining the binding specificity of RACK1 (Fig. 2B). The β -strand preceding the loop region contains the SIKIWD sequence, which is thought to be part of the PKC binding site on RACK1 (Ron and Mochly-Rosen, 1995). If this region in RACK1 were to contribute to binding PKC, then the outer β -strand of blade 6 would be required to adopt a nonblade conformation. Our model suggests that the lengthened loop could enable a degree of "conformational variability" in this region, with the outer strand of this propeller blade swapping in and out of blade conformation dependent on the presence of alternate binding partners.

Peptides from the V5 region of PKC have also been shown to contribute to RACK1 binding (Fig. 2A) (Stebbins and Mochly-Rosen, 2001). One of these, QEVIRN (amino acids 645–650 of PKC β II), shares amino acid homology with the outer β -strand of RACK1 blade 6, QEVIST (Fig. 2C); however, 3D structure is not currently available for this region of PKC. Our model presents the possibility that this region of PKC V5 could compete for and swap into the outer strand location on RACK1 blade 6. This model would also be consistent with an interaction between the C2 and V5 domains of PKC (Keränen and Newton, 1997; Stebbins and Mochly-Rosen, 2001; Banci et al., 2002). In molecular terms, this suggests that all these interactions could be mediated by exchange of β -strands within the β -sheet framework of blade 6.

Looking at the loop that separates the β -strands in blade 6 of RACK1, we see that the comparable loop in the PKC C2 domain mediates calcium binding and contributes three ligands provided by Asp-246, Asp-248, and Asp-254 (Fig. 2B) (Banci et al., 2002). Interestingly, these ligands align to acidic residues in the equivalent RACK1 region, when overall alignment is determined by SIKIWD mapping to SVEIWD. In addition, the glutamic acid in the QEVIRN sequence of the PKC V5 region maps to Asp-254 of PKC C2 (Fig. 2B). This raises the possibility that calcium ion binding may be involved in our suggestion of β -strand exchange for interactions within PKC (between C2 and V5), as well as between RACK1 and PKC. If such calcium involvement exists, it could, in principle, add an additional regulatory element or simply represent common interactions in each of the possible interacting combinations.

DIINALCF, one of the RACK1 sequences that has similarity to PKC-binding sequences, partly forms the inner β -strand in the RACK1 blade 6 model (Fig. 2B) (Ron and Mochly-Rosen, 1994). Our model for outer-strand, exchange-mediated interactions does not suggest a direct interaction between the inner strand and PKC. However, in the framework of strand exchange, it is possible that strand-forming potential alone could supply a measure of binding affinity, and we suggest that this could form the basis for some of the peptide binding data. All elements of this model, from RACK1 structure to binding partner conformations and calcium involvement, require testing with detailed biochemical and structural analysis.

Yeast two-hybrid screens have been used to identify a number of novel RACK1-interacting partners. An example of this is the cyclic AMP-specific phosphodiesterase PDE4D5. This is one of a large family of PDE isoforms (Houslay, 2001). The PDE4 family is encoded by four genes, each of which generates up to five isoforms that are distinguished by unique N-terminal regions (Houslay, 2001). The interaction

between RACK1 and PDE4D5 is extremely specific; PDE4D5 was not found to interact with various other WD-repeat proteins and RACK1 does not interact with any other PDE4 isoform (Yarwood et al., 1999). We have mapped the RACK1 interaction domain (RAID) in PDE4D5 to an 88-amino acid N-terminal region that is unique to PDE4D5 (Yarwood et al., 1999; Bolger et al., 2002). The predicted helical nature of the interaction site raises the possibility that the binding of PDE4D5 to RACK1 may occur in a manner analogous to the binding of G γ to the WD repeat protein G β (Fig. 4) (Steele et al., 2001). Mapping of the PDE4D5 interaction site on RACK1 by a combination of yeast two-hybrid and N-terminal deletion analyses demonstrated that WD repeats 5 to 7 of RACK1 are essential for it to interact with PDE4D5 (Steele et al., 2001). However, a RACK1 construct generated from these last three WD-repeats showed an interaction with PDE4D5 that was approximately 25% as effective as wild-type RACK1 (Steele et al., 2001). This may indicate a minimum core unit for PDE4D5 interaction. Whether this reduced interaction with PDE4D5 compared with RACK1 itself represents poor folding or a requirement for additional sequence to optimize interaction remains to be seen. In this regard, WD 1 is needed to complete the last blade of the propeller structure and loss of this might underpin the poor efficacy of the N-terminal truncate (Fig. 1A). Additionally, a reverse two-hybrid screen using these repeats identified 11 single, nonproline amino acid mutations within this region that nullify interaction with PDE4D5 (Steele et al., 2001). Mapping of these mutations onto our structural model of

RACK1 indicates that the amino acid residues essential for the RACK1/PDE4D5 interaction predominantly cluster on the same face of RACK1 (Fig. 5) (Steele et al., 2001). A large number of the mutations isolated in the screen were proline substitutions, which would be predicted to cause significant disruption of RACK1 folding. Indeed, our 3D-simulation of RAID bound to WD 5 to 7 of RACK1 demonstrates that these mutations occur at the blade interfaces for intact RACK1, indicating that their effect may not be mediated directly through RACK1-RAID interactions (Fig. 5). Because the mutations were isolated in the WD 5 to 7 construct, rather than the full propeller with its complement of blade interfaces, it is likely that stability of the individual blade structures may be particularly sensitive to amino acid changes. Intriguingly, many of the residues identified in RACK1 as being important for interaction with PDE4D5 are conserved in the primary structure of RACK1 from diverse species, including yeast (Fig. 6). However, the two phosphodiesterase genes in yeast show no indication of being PDE4 homologs and have no homology with the unique N-terminal region of PDE4D5 that directs interaction with RACK1 (Wilson and Tatchell, 1988; Matviw et al., 1993; Yarwood et al., 1999). Given the remarkable degree of conservation of these residues, it is possible that they are critical for the structural integrity and proper function of RACK1, perhaps by supporting the correct conformation of RAID binding sites.

The requirement of these residues for RACK1 function requires further clarification and is an important consideration when interpreting data from experiments involving

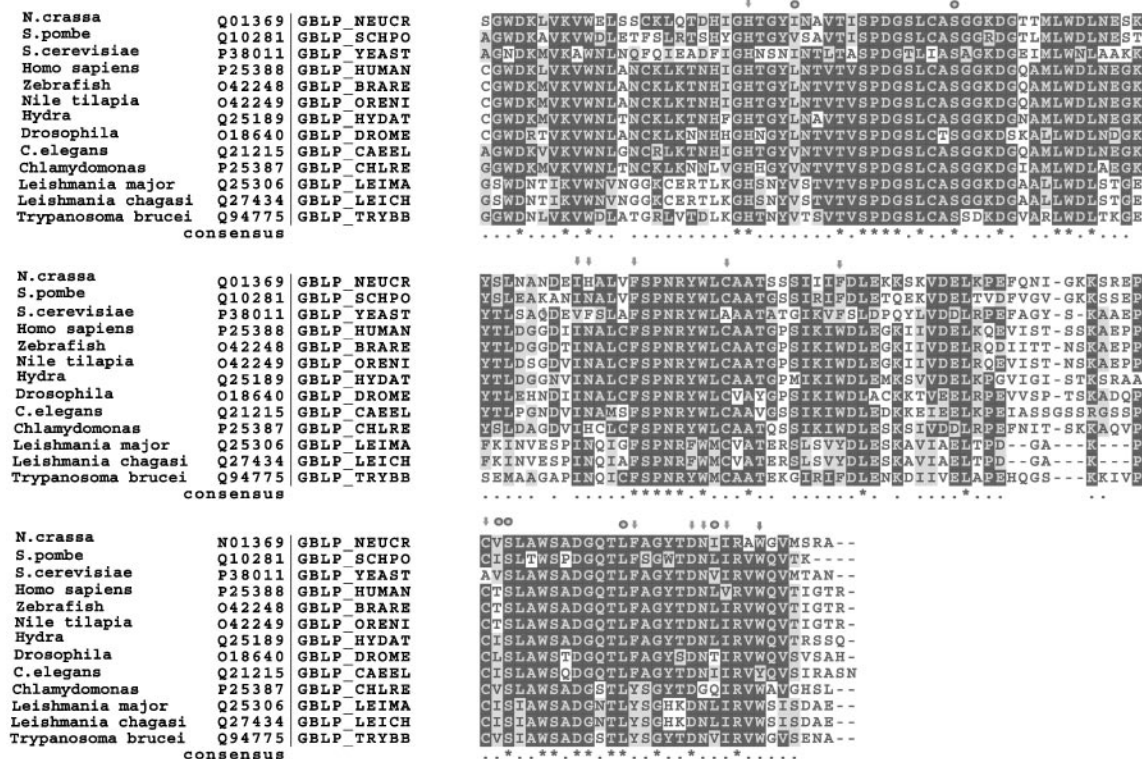


Fig. 6. Species conservation of binding mutations in the RACK1 WD5-7. A multiple alignment of the C-terminal portion of RACK1 from various species (left) is shown, together with their GenBank and SWISSPROT accession numbers, respectively. Sequence comparisons were constructed using CLUSTAL W (Thompson et al., 1994). The alignment output includes indicators that demonstrate the degree to which amino acids are conserved between RACK1 sequences from the different species. *, residues that are identical (completely conserved) throughout the stack; conservative and semiconservative (i.e., aliphatic) are indicated by colons (:) and periods (.), respectively. RACK1 mutations (Steele et al., 2001) that reduce RAID binding to human RACK1, and have been isolated more than once, are indicated on the top line of each cluster by a circle or arrow depending on whether residues were mutated to proline or to another residue, respectively.

over-expression of RACK1. For example, in the study by Buensuceso et al. (2001), alanine residues were introduced into WD 6 of RACK1 in the putative PKC-binding site (DIINALCF) of RACK1 that, when over-expressed in CHO cells, reversed inhibition of cell movement by wild-type RACK1. Although the authors claim that this effect is through disruption of PKC binding to RACK1, one of the residues converted to alanine was I236, a residue we now know to be essential for interaction with PDE4D5 (Buensuceso et al., 2001; Steele et al., 2001). Therefore, it could be argued that the effects observed in this study could also be caused by inhibition of PDE4D5 binding through overexpression of the mutant form of RACK1 or by disruption of RACK1 structural integrity.

3D-modeling analysis of the interaction between G_β and G_γ has led Sondek and Siderovski (2001) to propose that various proteins binding to the C-terminal region of β -propeller proteins may do so through a G- γ -like (GGL) motif. The cores of this are sequences DPLV and NPW (Fig. 3). They suggest that the N terminus of PDE4D5, because of due to the presence of similar motifs, might resemble a GGL domain and thus bind to the C-terminal region of RACK1 in a manner similar to the interaction between G_β and G_γ (Fig. 3). We have found that alignment of the amino acid sequences of PDE4D5 N terminus, the C2 domain of PKC β II and the C2 domains of other proteins reveals a remarkable degree of homology, particularly around the NPW motif of PDE4D5 (Fig. 3). Thus, by analogy with the GGL model, NPW may represent a common core motif as seen with homologous proteins. If this is the case, then the specificity of interaction must come from additional structural motifs that either enhance or reduce interaction with particular β -propeller proteins. The existence of this type of "structural conditioning" would mean that a particular family of β -propeller proteins could have different specificity with regard to their protein-binding partners. Additionally, for each β -propeller protein, there may be a family of proteins that can even interact at one 'site'. From the 3D models presented here (Figs. 1, 2, 4, and 5), we can see that WD 5 to 7 of RACK1 has a range of additional putative interaction sites along a bifurcated groove and therefore a range of possible modes of interaction, including those, like PKC, that interact only with part of the surface, and those, like RAID, that interact with multiple determinants over an extended surface.

RACK1 Signal Transduction

PKC. The in situ association and comovement of RACK1 and PKC β II has been demonstrated in CHO cells treated with phorbol 12-myristate 13-acetate, in NG108-15 neuroblastoma cells (Ron et al., 1999), and in cardiomyocytes (Ron et al., 1995). The localization of RACK1 was found to be very much cell-type dependent in both stimulated and unstimulated cells. That the localization of RACK1 alters concomitantly with PKC activation suggests that RACK1 does not anchor PKC β II to one place but is probably involved in the shuttling of the active enzyme to its appropriate subcellular site of action.

Introducing C2- or V5-region-derived peptides into cells can inhibit translocation of PKC and disrupt a variety of cellular functions, including *Xenopus leavis* oocyte maturation,

activation of PLD, and myocyte hypertrophy (Ron et al., 1995; Thorsen et al., 2000; Stebbins and Mochly-Rosen, 2001). These peptide inhibition studies raise the exciting possibility that peptide-mimetic small molecules could be generated that can specifically alter the function of RACK1-interacting proteins, with potential therapeutic benefit. Indeed, Rotenberg and Sun (1998) have reported that the PKC inhibitor DECA acts at the RACK1 binding site. Exposure of human breast adenocarcinoma cells to DECA resulted in reduced translocation of PKC α from the cytosolic to particulate fraction after phorbol ester stimulation, presumably because of the inability of PKC α to bind RACK1. The inhibition of RACK1-mediated translocation of PKC is thought to underlie the ability of DECA to delay morphological changes in fibroblasts in response to phorbol ester treatment, to inhibit cell motility and invasion, and to act as an antitumor agent. A potential problem underlying such studies is that inhibitor peptides and small molecules like DECA might be affecting the interactions between RACK1 and a number of different RACK1-binding partners because of homologies in their RACK1-interaction domains.

PDE4D5. The interaction of RACK1 with a critical regulator of cAMP metabolism suggests that RACK1 may be intimately involved in the regulation of pathways activated by adenylyl cyclase. Indeed, the adenylyl cyclase activator forskolin has been reported to cause RACK1 to localize to the nucleus, whereas PKC β II localization remains unaffected (Ron et al., 2000). RACK1 may therefore be involved in the shuttling of non-PKC protein binding partners to the nucleus and may play a role in cAMP-mediated gene expression. Another cAMP-elevating agent, ethanol, which increases the activity of adenylyl cyclase, thereby activating the cAMP/PKA signal transduction cascade (Saito et al., 1985), also induces the translocation of RACK1 to the nucleus (Ron et al., 2000). Ethanol also promotes the translocation of the catalytic subunit of PKA to the nucleus (Dohrman et al., 1996), and the ethanol-induced compartmentalization of RACK1 is blocked by adenosine-3',5'-cyclic monophosphorothioate, *Rp*-isomer, an inhibitory analog of cAMP that prevents the activation of PKA. These observations suggest that the recruitment of PDE4D5 to RACK1 may have a pivotal role in regulating the activity of the fraction of cellular PKA involved in regulating gene activity. Given the accumulation of evidence from yeast and mammalian cell systems these genes will possibly be those that are involved in the control of cell growth.

Intriguing new evidence suggests that RACK1 may, in certain circumstances, contribute to the regulation of collaborative interactions between PKC and cAMP signaling cascades. The chloride channel function of the cystic fibrosis transmembrane regulator (CFTR) plays a cardinal role in the control of humidity and electrolyte balance of conducting airways. The cAMP pathway, through the activation of PKA, tightly regulates the activity of the CFTR. Use of pharmacological agents has implicated PKC activation, particularly PKC ϵ , as a permissive requirement for cAMP-regulation of CFTR channel activity. The mechanisms underlying this phenomena are unclear because the physiological target of activated PKC has yet to be defined (Liedtke et al., 2002). It has been demonstrated, however, that RACK1 interacts with NHERF1, a CFTR-interacting protein (Liedtke et al., 2002). NHERF1 seems to act as a protein scaffold that brings the

CFTR, RACK1, and PKC ϵ together to enhance cAMP-control of CFTR. Inactivation of PKC ϵ , or displacement of PKC ϵ from its binding site on RACK1, would diminish cAMP-regulated CFTR function. It could also be imagined that recruitment of PDE4D5 to CFTR-associated RACK1 with the possible displacement of PKC ϵ would, by reducing local concentrations of cAMP, dramatically impair CFTR activity. Such a scheme may represent a novel CFTR desensitization mechanism and a possible site for therapeutic intervention.

Tyrosine Kinases/Phosphatases. In addition to interaction with Ser/Thr kinases such as PKA and PKC, RACK1 also liaises with cellular tyrosine kinases with possible growth-regulatory consequences. RACK1 was identified as a binding partner in a yeast two-hybrid screen to identify proteins that interact with Src tyrosine kinase (Chang et al., 1998). In vitro binding studies with glutathione *S*-transferase (GST) fusion proteins revealed that two other Src family tyrosine kinases, Lck and Fyn, also bind RACK1 and that RACK1 binds to the SH2 domain of Src (Chang et al., 2001). RACK1 and Src were shown to coimmunoprecipitate from CHO cells transfected with RACK1 and Src but not in cells cotransfected with RACK1 and a Src mutant that has a three-amino acid deletion in the phosphotyrosine-binding pocket of the SH2 domain, indicating that RACK1 interacts with the SH2 domain of Src in vivo. RACK1 and Src were also demonstrated to coimmunoprecipitate from NIH3T3 cells using either anti-RACK1 or anti-Src antibodies. The results of coimmunoprecipitations using mutant RACK1, in which each tyrosine has been individually substituted with phenylalanine together with phosphopeptide competition assays, suggest that Src interacts with phosphotyrosines in the sixth WD repeat of RACK1 (Chang et al., 2001). An in vitro protein kinase assay showed that GST-RACK1 could inhibit Src activity in a concentration dependent manner, although it had no effect on the activities of three Ser/Thr protein kinases (Chang et al., 1998). Levels of Src activity and tyrosine phosphorylation of many proteins were markedly reduced in cells overexpressing RACK1. Fibroblasts stably overexpressing RACK1 were observed to grow more slowly than wild-type cells. This lower growth rate in RACK1 overexpressing cells seems to have been caused by a prolongation of the G₀/G₁ stage of the cell cycle rather than an effect of necrosis or apoptosis. The authors propose that RACK1 exerts its effect on the growth of NIH3T3 cells via its inhibition of Src activity but acknowledge that this is only one of the possible mechanisms by which RACK1 may influence cell growth (Chang et al., 1998).

In addition to controlling cell growth processes, Src is known to be involved in brain functions such as learning, memory, and long-term potentiation, and also phosphorylates the NMDA ionotropic glutamate receptor. Interestingly, it has recently been found that the Src family-member Fyn binds to RACK1, which leads to the recruitment of Fyn to the ctNR2B subunit of the NMDA receptor and inhibition of its kinase activity (Yaka et al., 2002). Based on these observations and peptide displacement experiments, a model has been proposed whereby RACK1 mediated-recruitment, followed by release of Fyn, leads to phosphorylation of ctNR2B, thereby enhancing the activity of the NMDA receptor channel.

RACK1 has also been found to interact with the receptor protein tyrosine phosphatase PTP μ in a yeast two-hybrid

screen using the membrane proximal catalytic region of PTP μ as bait (Mourton et al., 2001). PTP μ has an intracellular domain with tyrosine phosphatase activity and an extracellular domain that is involved in cell adhesion via homophilic binding. Treatment of cells with phorbol esters has little effect on RACK1/PTP μ association; however, their interaction was found to increase at high cell density, suggesting that it is promoted by cell contact (Mourton et al., 2001). RACK1 and PTP μ have been shown to exist in a multiprotein complex with PKC δ in the developing neurites and growth cones of retinal explants (Rosdahl et al., 2002). Blockade of PKC activity with pharmacological inhibitors was found to inhibit outgrowth of neurites on a PTP μ substrate, providing circumstantial evidence that RACK1 is involved in the regulation of these processes (Rosdahl et al., 2002). Indeed, RACK1 is predominantly cytoplasmic in subconfluent cells, but when cell density increases, RACK1 translocates to regions of cell-cell contact to colocalize with PTP μ (Mourton et al., 2001). In cells infected with an antisense PTP μ retrovirus, RACK1 no longer localizes to points of cell-cell contacts (Mourton et al., 2001). Interestingly, constitutively active Src disrupts the interaction between RACK1 and PTP μ in a kinase-independent manner, suggesting that PTP μ and Src may compete to form mutually exclusive complexes with RACK1 (Mourton et al., 2001). RACK1 interacts with the conserved catalytic domain of PTP μ ; therefore, it may also interact with other PTPs, presenting the possibility that PTP versus PTK competition for binding to RACK1 may regulate other signaling complexes.

A degree of caution must therefore be applied when interpreting results derived from different experimental systems, because the ratio of RACK1-binding partners may vary dramatically in a cell-type specific manner, thereby affecting the signaling complexes that RACK1 is capable of interacting with. This may explain some apparently contradictory reports on the modulation of the MAPK pathway by RACK1 (Hermanto et al., 2002; Kiely et al., 2002). Overexpression of RACK1 in R⁺ fibroblasts and MCF-7 cells leads to enhanced activation of the extracellular signal-regulated kinase and c-Jun NH₂-terminal kinase mitogen-activated cascades, concomitant with an inhibition of protein kinase B, in response to IGF-1 stimulation (Kiely et al., 2002). In these cells RACK1 is in a complex with the p85 subunit of phosphatidylinositol-3-kinase and SHP-2. In contrast, in NIH-3T3 cells, RACK1 inhibits IGF-1-induced, β 1-integrin-associated kinase activity and association of Crk with p130CAS but has no effect on IGF-1-activated IRS-1, Shc, phosphatidylinositol-3-kinase, and extracellular signal-regulated kinase pathways (Hermanto et al., 2002). Clearly, a detailed analysis of the full range of signaling protein interactions that RACK1 is capable of mediating is required before these apparent discrepancies can be resolved.

RACK1 Cell Physiology

Cell Development. Homologs of RACK1 have been discovered in genetically malleable organisms such as *D. melanogaster* and yeast, providing an invaluable step toward elucidating its cellular functions. These investigations have begun to point toward a multifaceted role for RACK1 in cell physiological processes, which may be tailored to the requirements of individual cell types.

A fission yeast homolog of mammalian RACK1, cross-pathway-control (Cpc) 2, having 77% similarity with mammalian RACK1, was isolated in a yeast two-hybrid screen to identify proteins that interact with Pat1, a kinase that has no structural homolog in other organisms (McLeod et al., 2000). The life cycle choices of the fission yeast *S. pombe* are governed by nutritional signals and pheromone signaling and are regulated by several signal transduction pathways including the cAMP and MAPK pathways (Yamamoto et al., 1997). Each stage of the life cycle can be regulated by the activity of Pat 1 (McLeod et al., 2000). Activated Pat1 inhibits sexual differentiation in fission yeast, whereas its inactivation is necessary to initiate G₁ arrest, conjugation and meiosis (McLeod et al., 2000). Thus RACK1 may serve to assemble a "signalingosome" that includes Pat1 and, perhaps, other proteins that either regulate Pat1 or provide substrates for it. Certainly this interaction has functional significance, because mutant *S. pombe* lacking Cpc2 (Δ Cpc2 cells), while viable, display cell cycle abnormalities. These include facets associated with mitotic delay, cell elongation, and defects in conjugation and meiosis. Such cell cycle defects in Δ Cpc2 cells could be rescued by expression of Cpc2 and also by expression of mammalian RACK1, indicating that RACK1 and Cpc2 are indeed structural and functional homologs (McLeod et al., 2000). Such a system offers the opportunity of rescue with mutant forms of RACK1 that can be used to probe functional attributes associated with distinct WD-repeat structures.

In Δ Cpc2 cells, Pat1 kinase does not accumulate to high levels in the nucleus as it does in wild-type cells and instead displays a prominent, punctate cytoplasmic distribution (McLeod et al., 2000). Therefore, analogous to the situation with RACK1 and PKC β , Cpc2 may regulate Pat1 not by altering its catalytic activity but by influencing its subcellular localization. Disruption of Pat1 targeting in Δ Cpc2 cells may go some way to explaining why Cpc2 is not absolutely required for yeast development but is essential for the timing and progression of development. The phenotypes observed in cells lacking Cpc2 are similar to a subset of phenotypes observed in cells with defects in the stress-activated MAPK pathway and in cells expressing constitutively activated Pat1 (McLeod et al., 2000), suggesting that Cpc2 normally functions to regulate the activity of these pathways. The cell cycle, differentiation, and stationary phase defects of Cpc2-null mutants are phenotypes associated with high cAMP and activation of the PKA pathway (DeVoti et al., 1991; Mochizuki and Yamamoto, 1992). Because RACK1 has been demonstrated to interact with a cyclic AMP-specific phosphodiesterase isoform, PDE4D5, this prompted investigation as to whether Cpc2 was also involved in modulating cAMP signaling processes (Yarwood et al., 1999; McLeod et al., 2000). However, it seems that Cpc2 is not involved significantly in cAMP-regulated yeast cell processes, such as transcription of glucose- and nitrogen- sensitive genes or sexual differentiation and stationary phase survival. This is perhaps not surprising, because the two phosphodiesterase genes in yeast show no indication of being PDE4 homologs; they also have no homology with the unique N-terminal region of PDE4D5 that directs interaction with RACK1 (Wilson and Tatchell, 1988; Matviw et al., 1993; Yarwood et al., 1999).

Intriguingly, in both *D. melanogaster* and *X. laevis* zygotes, the RACK1 gene shows a dynamic expression pattern during maturation (Vani et al., 1997; Kwon et al., 2001). In addition,

examination of RACK1 protein during early embryonic development of chick limbs revealed that its expression is associated with proliferating cells of the limb mesenchyme and is further induced after treatment with fibroblast growth factor (Lu et al., 2001). This indicates that RACK1 may have a key role in regulating cell development, particularly in the regulation of cell proliferation and growth factor action. RACK1 protein may therefore play a cardinal role in the control of development, the true significance of which will only be revealed by gene disruption experiments.

Cell Movement and Growth. The use of yeast cell models has clearly demonstrated a link between Cpc2/RACK1 and cell cycle control. In recent years, a number of studies have focused on the role of RACK1 in cell growth control mechanisms in mammalian cells. Overexpression of RACK1 in NIH3T3 mouse fibroblasts has been found to cause a reduction in growth rate in both anchorage-dependent and -independent conditions because of a G₁ delay, which correlates with increased levels of the cyclin-dependent kinase inhibitors p21^{Cip1/WAF1} and p27^{Kip1} (Chang et al., 1998; Hermanto et al., 2002). In addition, cells that overexpress RACK1 demonstrate enhanced spreading, an increased number of actin stress fibers, focal contacts, and enhanced tyrosine phosphorylation of both focal adhesion kinase and paxillin (Buensuceso et al., 2001; Hermanto et al., 2002). Conversely, reduction of RACK1 expression in NIH3T3 cells by antisense depletion blocked cell spreading and inhibited growth factor-stimulated cell proliferation (Hermanto et al., 2002).

A yeast two-hybrid screen to identify proteins that interact with the cytoplasmic domain of β -integrins identified WD repeats 5 to 7 of RACK1, presenting further alluring evidence for an involvement of RACK1 in cell adhesion and movement (Liliental and Chang, 1998). Integrins are $\alpha\beta$ -heterodimeric cell surface receptors that mediate binding of cells to the extracellular matrix (ECM) (Skubitz, 2002). The ECM/integrins interaction induces signals required for reorganization of the actin cytoskeleton and formation of focal adhesion complexes, resulting in the activation of FAKs, the Src/MAPK pathway, increased intracellular calcium, activation of PKC, and alterations in cell transcriptional activity (Humphries, 1996; Yarwood and Woodgett, 2001). Full-length RACK1 was found to bind to β -integrins only upon phorbol ester treatment, a stimulus known to enhance integrin-mediated cell adhesion. The authors conclude that RACK1 may play a role in membrane-cytoskeletal association by acting as a scaffold to recruit other proteins to focal adhesion complexes (Liliental and Chang, 1998). One such protein might be PKC ϵ , which has been shown to be important for the control of integrin-dependent adhesion, spreading, and motility of human glioma cells (Besson et al., 2002). RACK1 has been shown to act as a protein adapter, linking PKC ϵ to integrin β chains (Besson et al., 2002). Disruption of the PKC ϵ targeting to integrin receptors, by antisense depletion of RACK1 or over-expression of a truncated form of RACK that lacks part of the integrin binding region (amino acids 204–317, containing WD repeats 6 and 7 and part of WD repeat 5), leads to impaired adhesion and migration of cells (Liliental and Chang, 1998; Besson et al., 2002).

One of the functions of RACK1, therefore, may be to control the interactions of signaling pathways involved in the coordination of cell adhesion, movement, and division. In addi-

tion, by controlling interactions with the ECM, RACK1 may also play an important role in governing cell survival. Consequently, RACK1 may play an important role in tissue remodeling processes such as wound healing. Certainly, RACK1 mRNA and protein is up-regulated in damaged and repairing segments of proximal kidney tubules within 12 h after acute ischemic renal injury in rats (Padanilam and Hammerman, 1997). A separate study identified RACK1 as being significantly up-regulated during angiogenesis and in carcinomas (Berns et al., 2000). Because PKC β signaling is known to play an important role in angiogenesis and tumor growth, it is suggested that the availability of RACK1 may be relevant to the downstream signaling of PKC β in angiogenically active tissues and may have a central role in tissue remodeling processes per se.

Immune Responsiveness. Ligand-initiated activation of superoxide anion generation by phagocytic cells such as neutrophils is a key mechanism in the immune response, and it has recently been proposed that PKC β II and RACK1 are involved in these critical functions (Korchak and Kilpatrick, 2001). Peptide inhibition of RACK1/PKC β II complex formation and antisense depletion of RACK1 were found to enhance superoxide anion generation in neutrophilic HL60 cells, suggesting that RACK1 may sequester PKC β II to negatively regulate superoxide anion generation or may divert PKC β II to other signal transduction pathways (Korchak and Kilpatrick, 2001). Intriguingly, almost all neutrophil inflammatory functions are susceptible to inhibitors of PDE4 cyclic AMP phosphodiesterase activity, the predominant PDE isoform in these cells (Zhu et al., 1998). Inhibition of PDE4 activity blocks oxygen radical release from neutrophils stimulated with a range of ligands, including tumor necrosis factor- α , *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine, and C5a, whereas the phagocytic respiratory burst is less susceptible (Souness et al., 2000). Therefore, a complementary mechanism underlying some of the effects of RACK1-antisense treated HL60 cells may function through the inappropriate intracellular targeting of PDE4D5, which is also known to interact with RACK1.

Further evidence linking RACK1 to immune responses is its association with cytokine and interferon receptors. Geijsen et al. (1999) have demonstrated a constitutive interaction between RACK1 and the common signaling subunit, the β -chain of the receptors for the hematopoietic and inflammatory cytokines interleukin-3 (IL-3) and IL-5 and granulocyte macrophage colony stimulating factor (GM-CSF). This interaction was discovered in a yeast two-hybrid screen using the β -chain as bait and was verified by coimmunoprecipitation and pull-down assays (Geijsen et al., 1999); however, the physiological significance of this interaction remains to be determined. Although stimulation with phorbol ester or IL-5 leads to increased association of PKC β with the receptor complex, it is not clear whether RACK1 mediates this interaction. RACK1 may therefore regulate other IL-3/IL-5/GM-CSF receptor signaling functions, for example activation of signal transducers and activators of transcription (Stats) 5a and 5b (Mui et al., 1995). Indeed, RACK1 has been linked to the activation of Stats after type I interferon receptor activation (Usacheva et al., 2001). IFNs α , β , and γ mediate innate immune responses to viral infection through IFN α R/IFN α R2 for IFN α and IFN β and IFN γ R/IFN γ R2 for IFN γ (Colonna et al., 2002). Stimulation of these receptors acti-

vates Janus protein kinases JAK1 and JAK2, which leads to the tyrosine phosphorylation of Stat1 and Stat2 (Darnell et al., 1994). RACK1 has been reported to act as an adaptor protein linking constitutively bound, nonphosphorylated Stat1 to the long β -subunit of the IFN α R (Colonna et al., 2002). This interaction is critical for normal Stat activation and the induction of an antiviral state by IFN in fibroblasts (Colonna et al., 2002). Further study will be required, however, to ascertain whether RACK1 is involved in the promotion of Stat activity by the IL-3/IL-5/GM-CSF and other cytokine receptors.

Brain Function. Levels of RACK1 are reduced by around 50% in the brains of aged rats compared with adult or middle-aged rat brains (Pascale et al., 1996). This is accompanied by a loss of PKC β translocation, suggesting that a depletion of RACK1 contributes to the functional impairment in PKC activity in aged rat brains. PKC isozymes are expressed at high levels in the brain and are thought to be important in memory and learning processes (Selcher et al., 2002). In particular, conventional PKCs are involved in the regulation of a number of processes, such as neurotransmitter release, receptor desensitization, ion channel flux, and synaptic efficiency, which are known to undergo age-related modulation (Battaini et al., 1997). Intriguingly, the pathophysiology of Alzheimer's disease (AD) has been reported to involve attenuated PKC activity and translocation (Masliah et al., 1991; Matsushima et al., 1996). RACK1 levels are decreased in both soluble and membrane fractions from the brains of persons with AD, whereas PKC β II levels are unchanged (Battaini et al., 1999), suggesting that the impaired PKC signal transduction pathway in brains of persons with AD is related to a reduction in RACK1 protein levels. Somewhat confusingly, an earlier article by Shimohama et al. (1998) reported that RACK1 levels were not significantly affected in brains of persons with AD, but this discrepancy may reflect a difference in the brain areas examined.

RACK1 protein levels are also modulated in parallel with levels of PKC α and β in the brains of morphine-treated rats (Escriba and Garcia-Sevilla, 1999). Opiate drugs control the protein expression levels of conventional PKC isozymes in the brain, which may affect the activity of adenylyl cyclase, a principle mediator of opioid receptor signaling (Zhou et al., 1994; Busquets et al., 1995; Ammer and Schulz, 1997). This strong positive correlation between the levels of RACK1 and both PKC α and β has not been found for other proteins involved in opioid signal transduction, such as G α , G β , GRK2, adenylyl cyclase, and PKA (Nestler and Tallman, 1988; Terwilliger et al., 1994; Escriba and Garcia-Sevilla, 1999). This correlation suggests that morphine regulates the levels of cPKC and RACK1 in the brain by a co-ordinate mechanism and that RACK1 may be involved in the mechanisms of opiate addiction and withdrawal.

Another study (Ron et al., 2000) has also implicated RACK1 in the mechanisms of drug dependence. The exposure of both cultured cells and whole mouse brain to ethanol resulted in the uncoupling of PKC β II from RACK1 and provoked movement of RACK1 to the nucleus, whereas the compartmentalization of PKC β II remained unaffected (Ron et al., 2000). In vivo exposure to ethanol also causes the nuclear localization of RACK1 in specific regions of mouse brain, whereas PKC β II localization is unchanged. Chronic exposure to ethanol is known to result in neuroadaptive changes such

as tolerance, craving, and physical dependence, which are thought to be modulated to some extent by the alteration by ethanol of the action of PKC on some of the major neurotransmitters, including GABA, glutamate, glycine, and muscarinic receptors (Weiner et al., 1994; Dildy-Mayfield and Harris, 1995; Larsson et al., 1995; Mascia et al., 1998). Some of these neuroadaptive changes may therefore be associated with the ethanol-induced translocation of RACK1 to the nucleus.

Both PKC β II and RACK1 have been demonstrated to associate with GABA_A receptor β -subunits (Brandon et al., 1999). The amount of PKC β II associated with β 1/ β 3 subunits is dramatically increased by phorbol ester treatment, suggesting that it is activated PKC that is targeted to GABA_A receptors in neurons. PKC is known to phosphorylate GABA_A receptors and inhibit their function. That the PKC β II/ β -subunit interaction is direct shows that it is independent of RACK1; however, RACK1 may play an auxiliary role by modulating the affinity of interaction between PKC β II and GABA_A receptors or an action of PKC other than controlling receptor phosphorylation. In this respect, blocking PKC/RACK1 interaction disrupts the modulation of GABA_A currents by 5-HT₂, suggesting that RACK1-mediated targeting of PKC to the vicinity of GABA_A receptors is required for serotonergic signaling (Feng et al., 2001). Whether these interactions play a greater role in the wider mechanisms of drug dependence remains to be determined.

Conclusions and Future Perspectives

The multiplicity of cell functions in which RACK1 has been implicated probably reflects the ability of this scaffold protein to interact with a wide range of signaling proteins. Analogous to Cpc2 in yeast cells, mammalian RACK1 isoforms seem to direct "cross-pathway-control" by integrating communication from different signaling pathways through the orchestration of protein-protein interactions.

The wide range of vital cell processes that involve RACK1 suggests that studies into the function of this scaffold protein will continue to form the basis of an exciting and burgeoning research field. The question still remains, however, as to the specific combinations of RACK1-interacting signaling proteins that control individual cell functions. Many of the signaling proteins that bind to RACK1 target the C terminus of the protein (Fig. 1B). This suggests that in intact cells, there may be a degree of competition between signaling proteins for interaction with RACK1. This implicates RACK1 as contributing to the regulation of the balance of activation between conspiring or antagonistic signaling pathways. Alternatively, individual proteins may bind RACK1 at discrete intracellular sites. Implicit to this is the suggestion that it is the intracellular targeting of interacting partners, rather than RACK1, that is central to this. Thus PDE4D5, which is a predominantly cytosolic enzyme, will compete for binding to a different pool of RACK1 than activated PKC, which is targeted to particulate structures in cells (Yarwood et al., 1999). Fine structural detailing of the interaction interfaces between RACK1 and its various binding partners will lead to the development of highly specific protein complex disruption compounds that will facilitate the identification of cell functions linked to particular intracellular pools of RACK1. Because of their specificity, these compounds are predicted to possess potent therapeutic potential.

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